ISSN 1927-646X (Online)

Journal of Mosquito Research

Mosquitoes, Flies Making Troubles to Humans

ISSN 1927-646X
14004
9 771927 646008 >

2014

JMR Vol 4

Publisher
Sophia Publishing Group

Edited by
Editorial Team of Journal of Mosquito Research
Email: edit@ijms.biopublisher.ca
Website: http://jmr.biopublisher.ca

Address:
11388 Stevenston Hwy,
PO Box 96016,
Richmond, V7A 5J5, British Columbia, Canada

Journal of Mosquito Research (ISSN 1927-646X) is committed to publishing and disseminating all the latest and outstanding research articles, letters and reviews in all areas of mosquito research and the related field. All papers chosen for publications should be creative reviews and opinions or innovative research work in the field of mosquito and its affecting the living organisms, as well as environmental risk and public policy relevant to mosquito modified organisms. Topics include the research at molecular or protein level of mosquito, impact on the ecosystem, containing positive and negative information, natural history of mosquito, also publishing innovative research findings in the basic and applied fields of mosquito and novel techniques for improvement, as well as the significant evaluation of its related application field.

The primary criterion for publication is new insights that are not only of broad interest to scientists, and teachers/students/researchers engaged in mosquito research, but are also appropriate for R & D personnel as well as readers interested in mosquito research.

BioPublisher, operated by Sophia Publishing Group (SPG), is an international Open Access publishing platform that publishes scientific journals in the field of life science. Sophia Publishing Group (SPG), founded in British Columbia of Canada, is a multilingual publisher.

Latest Content

Journal of Mosquito Research, 2014, Vol.4, No.10
ISSN 1927-646X
http://jmr.biopublisher.ca

Research Report Open Access

Larvicidal Activity of *Nelumbo nucifera* Gaertn. (Nymphaeaceae) against *Anopheles stephensi* (Liston 1901) and its Effect on Non-target Organisms

Anushree Singha Ray, Kuntal Bhattacharya, Aniket Singh and Goutam Chandra
Mosquito, microbiology and nano-technology research units, Parasitology laboratory, Department of zoology, The University of Burdwan, Golapbag, Burdwan, West Bengal, Pin- 713104, India

Corresponding author email: goutamchandra63@yahoo.co.in
Journal of Mosquito Research, 2014, Vol.4, No.10 doi: 10.5376/jmr.2014.04.0010

Abstract

Background and objective: Control of mosquito vector is of paramount significance to reduce disease incidences as proper vaccines against mosquito borne diseases are yet to develop. Standard amalgamation of cost effective, target specific and bio-degradable insecticides is necessary owing to vector resistance, an evolutionary phenomenon and intoxication of nature through indiscriminate use of chemical insecticides. The present study projected larvicidal activities of the crude and solvent extracts of *Nelumbo nucifera* seed coats against the malarial vector *Anopheles stephensi* under laboratory conditions.

Methods: Crude extracts of *N. nucifera* seed coats were scrutinized for larvicidal activity against 1st to 4th instars larvae of *A. stephensi*. Solvent extractions of the same material fractions were carried out by means of petroleum ether, ethyl acetate and water. Dose dependent mortality assay was performed by graded concentrations (from 60 ppm to 180 ppm) of the solvent extracts. Quantifications of LC_{50} and LC_{90} values were consummated through log-probit analyses. ANOVA analyses were performed for statistical justifications of the larvicidal property. Impacts of the extracts on a non-target water fauna were evaluated under laboratory conditions.

Result: The highest mortality was recorded in 0.5% concentration of crude extract after 72 hours of post exposure. Ethyl acetate extract was found to be the most potent larvicidal agent exerting efficient larvicidal activity amongst all the three solvent extracts. Following introduction of ethyl acetate extract, 1st and 2nd instars larvae exhibited 100 per cent mortality within 72 hours of post-exposure. The mortality rates for 3rd and 4th instars were 96 and 72 per cent respectively with the same observation period. The rate of mortality (Y) was found to be positively correlated with the concentration (X) through regression analyses. ANOVA analyses established the statistical significance of the larvicidal activity of the seed coat extractives. However, the non-target populations were entirely non-responsive to extracts under study.

Conclusion: Seed coat extracts of *N. nucifera* is effective against *A. stephensi* larvae. It is non-toxic to non-target organisms and environment-friendly.

Keywords *Nelumbo nucifera;* Seed coat; Larvicidal*; Anopheles stephensi;* Non-target organism

1. Introduction

Amongst all arthropods, mosquitoes are the most abundant blemished insect for spreading many noxious tropical and subtropical diseases like filariasis, yellow fever, malaria, dengue, different types of encephalitis like Japanese encephalitis and many more diseases resulting millions of deaths yearly. Apart from mortality, mosquito bites result into angioedema, intense itching, redness of skin and swellings. Disease transmission path includes broad regions around the equator especially much of sub Saharan Africa, Asia and the Americas. *Anopheles stephensi* spread the noxious disease malaria in urban areas mainly. WHO has reported 219 million malarial cases with 1.2 million deaths in 2010 (Nayyar et al., 2012). There are about 10,000 malaria cases per year in Western Europe and 1300–1500 in the United States (Taylor *et al.*, 2012). Rainfall, warm temperatures and stagnant water bodies provide ideal habitats for mosquito larvae, so, malaria is prevalent in tropical and sub tropical regions. In urban area *An. stephensi* chooses different water-bodies for breeding, primarily in overhead tanks, artificial containers, walls and ground level water tanks (Jeyabalan *et al.*, 2003). Till now many malarial

cases are unreported and precise data is unavailable in many rural areas.

To prevent such types of malevolent diseases and to reduce fatality thereof, it is indispensable to find a way to control the mosquito population. For the purpose of vector control plentiful simulated insecticides have been developed and used against the vector and achieved a significant success, but it contains some alarming drawbacks like non-selectiveness and non-biodegradability which leads to toxic hazards (Wattal *et al*., 1981). Nevertheless indiscriminate use of commercial insecticides results genetic resistance (Devine, 2007) to pesticides among vector species, arbitrary use of those results some adverse effect on ecosystem, environment and to human health as well (Lee *et al*., 2001). For long term applications it is more difficult to rely upon the obtainable mosquitocidal agents after the outcome of bio pesticide resistance (Tabashnik, 1994), so, finding of eco-friendly and target specific insecticides specially from plant origin (Rawani *et al*., 2009; Chowdhury *et al*., 2009; Chakraborty *et al*., 2013) are apparent in a standard manner.

Nelumbo nucifera, the holy lotus found throughout India is a large aquatic medicinal plant with stout, creeping rhizome. Anti-diarrheal and antimicrobial properties (Mukherjee, 2002) of *N. nucifera* have been reported. Anti-inflammatory and anti-tumour effects; mediated by the presence of betulinic acid, a steroidal pentacyclic triterpenoid of this plant are also well documented (Mukherjee *et al.* 1997; Chou *et al*., 2000). This plant is well recognized for antioxidant (Ling *et al.*, 2005) anti-arrhythmic (Yu and Hu, 1997) properties.

Though diverse parts of *N. nucifera* have been stepped to discover several medicinal inimitabilities, seed coat has not yet been reported to contain mosquitocidal agents.

2. Material and Methods

2.1 Assemblage of plant materials

Fresh seedpods of *Nelumbo nucifera* (Nymphaeaceae) were collected randomly from the local ponds of Burdwan (23°16´N, 87°54´E), West Bengal, India during September to November 2012. The voucher specimen was numbered (voucher no. GCASR-03) and kept as a herbarium in the Department of Zoology, The University of Burdwan.

2.2 Nurturing the larvae and colony set up

Larvae of *An. stephensi* were collected from Kolkata and for further bioassay experiments larval colony is maintained under laboratory condition. The larvae were periodically fed with a diet containing powder of Brewer yeast, dog biscuits and algae mixture in 3:1:1 ratio (Kamaraj *et al.,* 2011a) and kept in plastic trays filled with tap water in a germ free condition. Pupae were collected from the trays and transferred to insectary (45×45×40 cm) for adult immergence. During the rearing of adults, they continuously provided a 10% sucrose solution with multivitamin syrup in a container with a cotton wick. The cotton was changed every day and kept moist with the solution. The mosquitoes were identified by the identification keys of Christophers (1933), Barraud (1934) and Chandra G (2000). In a different glass cage, the adults of *An. stephensi* were reared, on the 5th day; adults were given a blood meal from a non-motile shaved pigeon resting on the cages overnight for blood-feeding by females. Glass Petri dishes filled with 100 ml of tap water were kept inside the cage for oviposition. The eggs were unperturbed and allowed to hatch under laboratory conditions. In laboratory the colony of *An. stephensi* were maintained by repeating the process again and again. Mosquito colony was kept at 27±2°C, 75–85% relative humidity (RH), with a photoperiod of 14:10 h light/dark cycle.

2.3 Crude extracts procurements

Fresh and young seed coats of *N. nucifera* were rinsed in tap water followed by distilled water and soaked on a paper towel. Then the unspotted seed coats were minced by electric grinder and the liquid was filtered by Whatman’s no-1 filter paper. The filtrate solution was considered as the stock crude solution (100% concentration) and stored in refrigerator for future use.

2.4 Differential solvent extraction

For solvent extraction, fresh and clean seed coats of *N. nucifera* were dried for few days in shed. 120 g dried

seed coats of *N. nucifera* were put into the 'thimble' of the Soxhlet apparatus while 1200 ml solvent was loaded into the 'still pot' following 1:10 ratio. Three different solvents in a non-polar to polar fashion viz. petroleum ether, ethyl acetate and water were passed through the column one after another. The extraction phase was set for 8 hours a day with a maximum extraction period of 72 hours for a particular solvent. The extracts were collected from solvent chamber and concentrated through evaporation in a rotary evaporator and the residue obtained was stored at 4°C in a refrigerator.

2.5 Dose dependent larvicidal bioassay

Trusting the WHO standard protocol (WHO/ VBC, 1981) the larvicidal bioassay was executed at the Mosquito, Microbiology and Nano Technology Research Units, Parasitology Laboratory, The University of Burdwan. Instars wise 25 larvae of *A. stephensi* were affianced in Petri-dishes of 9 cm diameter (150 mL capacity) filled with 100 mL of tap water. From 0.1% to 0.5% crude extractives were added in different Petri dishes while 60 ppm to 180 ppm solvent extracts was given in different Petri dishes for performing larvicidal bioassay. Each experiment was performed in triplicate (n=75) with a set of controls. Petri dishes were retained at room temperature (29 ± 2°C), within 88±2% relative humidity range for a total observation period of 72 hours. The larvae were considered dead when they didn't exhibit any movement by the pricking with a sharp needle in the siphon or cervical region or they were unable to achieve the water surface **(**Macedo *et al*., 1997**).** The no. of dead wrigglers was calculated every 24 hours period up to 72 hours and percentage mortality was recorded from the average value of three replicates. The mortality of 48 h and 72 h were expressed by additions of the mortalities of 24 h and 48 h, respectively. Initially all the solvent extractives were screened against 3rd instars larvae and then the experiment was elaborated with the best active fraction against all the instars.

2.6 Effects on non-target populations

The little creatures sharing the same environment with mosquitoes are considered as the most fatal risk group. Vulnerability of these organisms to seed coat extractives was figured out on *Chironomus circumdatus* larvae (insect). They were exposed to concentration level of LC_{50} value (72 hours of post-exposure) of 3rd instars larvae to examine the mortality and other irregularities such as tardiness of swimming activity up to 72 h of exposure.

2.7 Statistical analyses

The percentage mortalities (%M) were precised by Abbott's formula (Abott WS, 1925) during the observation of larvicidal potentiality of the seed coat extracts. Judicious determination of LC_{50} and LC_{90} values of crude and solvent extracts were carried out through Log-probit and regression analyses. Further statistical calculations were done through ANOVA analyses using instars, hours of exposure and concentrations as three random variables to validate the significance between the above parameters and larval mortality.

3. Results

Different corporal characteristics and percentage yields for each solvent were significantly different from the other solvents as shown in Table 1. Larval mortalities at different concentrations of crude extractives of *N. nucifera* seed coats against *An. stephensi* wrigglers were presented instars wise in Table 2. The mortality percentages using all the three different extractives against 3rd instars larvae were presented in Figure 1. Ethyl acetate extract was found to be the choicest larvicide, through several trials, amongst all the extracts of *N. nucifera* seed coats. The larval mortality of *An. stephensi* using ethyl acetate extractives was depicted in Table 3. Log probit analyses clearly indicated augmentations in LC_{50} and LC_{90} values (at 95% confidence level) vis-à-vis late stages larvae which were subsequently subordinated with increase in post-exposure period (from 24 hours to 72 hours) (Table 4). All through the experiment the concentration of ethyl acetate extractives were found to be positively correlated with rate of mortality having a regression coefficient nearly 1. Further analyses using completely randomized three way ANOVA regarding concentration (C), hour (H) and instars (I) as three parameters revealed the statistical significance of the larvicidal effect of ethyl acetate extractive against *An. Stephensi* (Table 5). The non-target populations were found in normal condition after 72 h of post-exposure.

Table 1 Differential yields and some physical characters of *N. nucifera* seed coat extracts

Solvent extract	Appearance	Colour	Solubility	pH	Odor	Yield
Petroleum ether	Liquid	Greenish-yellow	Soluble in DMSO	6.62	Odorless	8.65 g
Ethyl acetate	Semi-solid	black	Soluble in acetone, absolute alcohol, water	6.94	offensive	9.20 g
Water	Liquid	Brownish yellow	Soluble in absolute alcohol	7.24	offensive	2.03 g

Table 2 Percent mortality of *An. stephensi* larvae using crude extracts of *N. nucifera* seed coats

Larval Instars	Concentration (%)	Mortality rate (Mean ±SE)		
		24h	48h	72h
1st	0.1	29.32 ± 1.25	33.33 ± 0.82	41.33 ± 0.94
	0.2	37.32 ± 1.63	45.33 ± 1.41	52.00 ± 0.82
	0.3	50.67 ± 2.05	60.00 ± 0.00	64.00 ± 0.94
	0.4	68.00 ± 1.63	73.33 ± 0.94	80.00 ± 1.47
	0.5	78.67 ± 0.80	82.67 ± 0.65	84.00 ± 0.00
2nd	0.1	24.00 ± 0.94	32.00 ± 0.82	36.00 ± 1.70
	0.2	34.67 ± 2.05	40.00 ± 1.63	42.67 ± 0.65
	0.3	52.00 ± 2.16	56.00 ± 2.05	60.00 ± 0.82
	0.4	69.33 ± 1.70	72.00 ± 0.82	74.67 ± 1.25
	0.5	73.33 ± 1.41	77.33 ± 0.47	80.00 ± 0.00
3rd	0.1	22.67 ± 0.00	29.33 ± 0.80	34.67 ± 1.47
	0.2	32.00 ± 0.94	37.33 ± 1.25	40.00 ± 2.45
	0.3	49.33 ± 0.00	53.33 ± 1.41	57.33 ± 0.00
	0.4	66.67 ± 1.94	69.33 ± 1.25	74.67 ± 1.41
	0.5	70.67 ± 1.63	73.00 ± 0.00	78.67 ± 0.00
4th	0.1	0.00 ± 0.00	8.00 ± 1.25	24.00 ± 0.00
	0.2	2.67 ± 1.25	9.33 ± 0.47	30.67 ± 0.00
	0.3	4.00 ± 0.00	13.33 ± 2.87	34.67 ± 0.47
	0.4	5.33 ± 0.47	17.33 ± 1.45	36.00 ± 0.82
	0.5	6.67 ± 1.28	21.33 ± 1.63	41.33 ± 2.16

Table 4 Assessment of LC_{50} and LC_{90} values of ethyl acetate extract of *N. nucifera* through log-probit and regression analyses

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2- value
1st	24	54.62	261.56	0.07 x + 9.86	0.96
	48	37.57	179.53	0.05 x + 14.06	0.99
	72	34.09	100.44	0.05 x + 16.73	0.95
2nd	24	73.34	496.21	0.06 x + 8.60	0.99
	48	47.96	382.15	0.05 x + 11.46	0.99
	72	43.74	143.46	0.06 x + 13.13	0.97
3rd	24	105.36	459.26	0.08 x + 3.27	0.95
	48	78.72	301.85	0.07 x + 6.8	0.95
	72	50.27	167.34	0.07 x + 11.86	0.97
4th	24	129.65	972.90	0.06 x + 4.67	0.99
	48	94.98	914.00	0.05 x + 7.87	0.97
	72	79.33	755.30	0.06 x + 8.27	0.96

Note: x = concentration (in ppm) of solvent extracts.

Table 3 Percent mortality of *An. stephensi* larvae using ethyl acetate extracts of *N. nucifera* seed coats

Larval Instars	Concentration ppm (mg/L)	Mortality rate (Mean ±SE)		
		24 h	48 h	72 h
1st	60	56.00 ± 0.94	68.00 ± 0.94	77.33 ± 0.82
	90	61.33 ± 1.63	74.67 ± 2.05	84.00 ± 2.95
	120	76.00 ± 2.05	80.00 ± 1.70	93.33 ± 0.94
	150	82.67 ± 1.63	86.67 ± 0.94	97.33 ± 1.25
	180	86.67 ± 0.80	92.00 ± 0.65	100.00 ± 0.00
2nd	60	49.33 ± 0.80	57.33 ± 0.82	68.00 ± 0.82
	90	54.67 ± 1.25	67.67 ± 1.63	77.33 ± 2.87
	120	62.67 ± 2.16	72.00 ± 1.41	80.00 ± 0.82
	150	69.33 ± 1.25	77.33 ± 0.81	89.33 ± 1.25
	180	77.33 ± 1.41	84.00 ± 0.47	100.00 ± 0.00
3rd	60	36.00 ± 0.00	41.33 ± 0.47	61.33 ± 1.47
	90	41.33 ± 0.82	58.67 ± 1.25	74.67 ± 2.45
	120	48.00 ± 0.00	66.67 ± 1.25	80.00 ± 0.00
	150	66.67 ± 1.94	73.33 ± 1.25	85.33 ± 0.47
	180	73.33 ± 0.94	80.00 ± 0.00	96.00 ± 0.00
4th	60	32.00 ± 1.92	41.33 ± 0.00	44.00 ± 0.82
	90	42.67 ± 0.47	50.67 ± 0.47	54.67 ± 2.47
	120	48.00 ± 0.00	56.00 ± 1.28	62.67 ± 0.47
	150	54.67 ± 0.47	61.33 ± 1.45	65.33 ± 0.82
	180	62.67 ± 0.47	65.33 ± 0.47	72.00 ± 0.82

Table 5 Completely randomized three way ANOVA analysis using concentration (C), hour (H) and instars (I) as three parameters

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean of squares (MS)	F value	p-level
Instars (I)	1079.91	3	359.97	285.44	0.00
Hours (H)	670.08	2	335.04	265.67	0.00
Conc. (C)	1223.02	4	305.76	242.45	0.00
I × H	50.99	6	8.50	6.74	0.00
I × C	31.42	12	2.62	2.08	0.02
H × C	15.31	8	1.91	1.52	0.16
I × H × C	20.51	24	0.85	0.68	0.86
Within groups	151.33	120	1.26	---	---
Total	3242.58	179	18.12	----	---

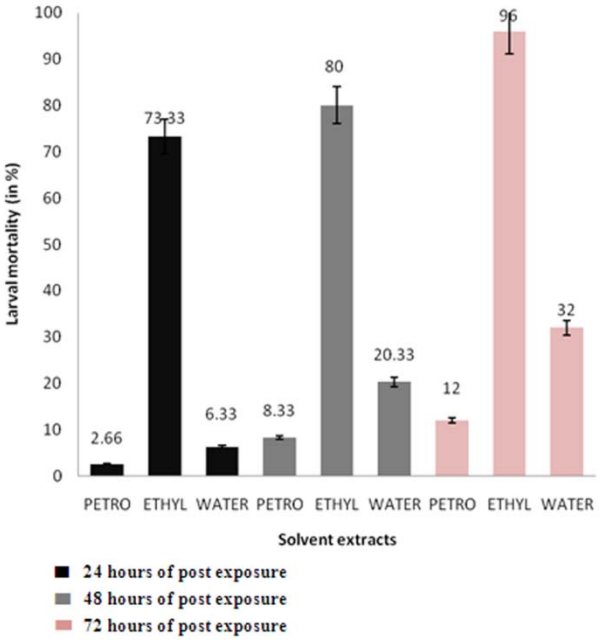

Figure 1 Graphical presentation of the larval mortality using all the three different extracts against 3rd instars larvae of *An. stephensi*

4. Discussion

Incapability to control of mosquito borne diseases remains a big dispute even today and indeed most challenging. Though personal protection from mosquito bites through several means are always advised, it is very little to control the menace. The best approach to shrink mosquito population is wrigglers' control. Employment of cost-effective, biodegradable natural insecticides from botanicals has given importance, recently, for this purpose. Mosquito larvicidal potentiality of several plants is verified by a number of workers (Singha *et. al*., 2012; Bhattacharya and Chandra 2013, 2014; Kundu et al., 2013). Plant constituents are well established as pupicidal (Rawani et al., 2012), repellent, adulticidal and smoke toxic (Singha *et al*., 2011, Chowdhury *et al*., 2007) against different mosquito species. The present study well conferred the target specific larvicidal activity of *N. nucifera* seed coats against *An. stephensi*. Govindarajan reported that methanol extract of *Andrographis paniculata*, *Eclipta alba*, and *Cardiospermum halicacabum* are very much effective against the larvae of *An. stephensi* with the median lethal concentration values of 79.68, 112.56, and 133.01 mg/L respectively. However, we found that the LC_{50} value of 3rd instars larvae was 50.27 mg/L (ppm) using ethyl acetate extracts of *N. nucifera* seed coat. Adhikari *et al*. (2012) reported the larvicidal activity of glacial acetic acid, an organic acid, against *An. stephensi*. They recorded 100% mortality of 3rd instars in 0.5% solution. Ethyl acetate extracts of *Annona squamosa* bark was reported to be larvicidal against the 4th instars larvae of *An. stephensi* (Kamaraj *et. al*., 2010) where LC_{90} values varied in between 70.38-210.68 ppm. *N. nucifera* leaf extract using methanol as solvent was found to be mosquitocidal against early 4th instars *An. stephensi* larvae (Kamaraj *et. al.*, 2011b) with the median lethal concentration 37.49 ppm following a period of 48 hours. We noticed the LC_{50} value of 94.98 ppm against mature 4th instars of *An. stephensi* larvae when the observation period was set at 48 hours. Ethyl acetate extracts of *N. nucifera* seed coats showed very promising responses against the 1st and 2nd instars of *An. stephensi* with the median lethal concentration values of 34.09 ppm and 43.74 ppm respectively. Entire larval populations of 1st and 2nd instars were diminished following a post-exposure period of 72 hours with no significant detrimental effect on non-target population. This ascertained the selective toxicity of the extract under study with meticulousness. High mortality percentage against 3rd instars and a moderate mortality value against 4th instars (table-3) pertained to the suitability of this active fraction as mosquitocidal bio-pesticide against *An. stephensi.*

In concise, the findings of the present study reveal that the seed coat extracts of *N. nucifera* posses larvicidal activity against *An. stephensi*. Other laboratory investigations are mandatory for enlightening the actual chemical amalgam responsible for larvicidal activity. This veteran activity may again be evaluated against *An. stephensi* larvae in field conditions. For successfully utilization of this novel mosquitocidal source through commercial formulation detail research is necessary.

Conflict of interest statement

We pronounce that we don't have any conflict of interest.

Acknowledgements

The authors are indebted to Professor Dr. A. Mukhopadhyay, Botany Department, The University of Burdwan, for his kind help in plant species identifications. We are grateful to UGC DRS for providing financial assistance.

Reference

Abott W.S., 1925, A method of computing the effectiveness of an insecticide, J. Econ. Enotomol., **18**(2): 265-267

Adhikari U., Ghosh P., and Chandra G., 2012, Biocontrol efficacy of an organic acid against larval form of *Culex quinquefasciatus*, *Culex vishnui* group and *Anopheles stephensi*, Asian. Pac. J. Trop. Dis., 485-487

Barraud P.J., 1934, The Fauna of British India, including Ceylon and Burma, Diptera Vol -IV. Taylor and Francis London: pp.1-455

Bhattacharya K., and Chandra G., 2013, Bioactivity of *Acyranthes aspera* (Amaranthaceae) Foliage against the Japanese Encephalitis Vector *Culex vishnui* Group, J. Mosq. Res., **3(13)**: 89-96

Bhattacharya K., and Chandra G., 2014, Phagodeterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata* L. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus* (Diptera: Culicidae), Asian. Pac. J. of Trop. Dis., **4(Suppl 1)**: S226-S232 http://dx.doi.org/10.1016/S2222-1808(14)60444-8

Chakraborty S., Singha S., Bhattacharya K., and Chandra G., 2013, Control of human filarial vector, *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) through bioactive fraction of *Cayratia trifolia* leaf, Asian. Pac. J. Trop. Biomed., **3(12)**: 980-984 http://dx.doi.org/10.1016/S2221-1691(13)60189-6

Chandra G., 2000, Mosquito, Sribhumi Publication Co. pp.1-102

Chou K.J., Fang H.C., Chung H.M., Cheng J.S., Lee K.C., Tseng L.L., Tang K.Y., and Jan C.R., 2000, Effect of betulinic acid on intracellular-free Ca^{+2} levels in Madin Darby canine kidney cells, Eur. J. Pharmacol., **408(2)**: 99–106 http://dx.doi.org/10.1016/S0014-2999(00)00750-0

Chowdhury N., Chatterjee S.K., Laskar S., and Chandra G., 2009, Larvicidal activity of *Solanum villosum* Mill (Solanaceae: Solanales) leaves to *Anopheles subpictus* Grassi (Diptera: Culicidae) with effect on non-target *Chironomus circumdatus* Kieffer (Diptera: Chironomidae), J. Pest. Sci**., 82**: 13-18 http://dx.doi.org/10.1007/s10340-008-0213-1

Chowdhury N., Bhattacharjee I., Laskar S., and Chandra G., 2007, Efficacy of *Solanum villosum* Mill. (Solanaceae: Solanales) as a biocontrol agent against fourth instar larvae of *Culex quinquefasciatus* Say, Turk. J. Zool., **31(4)**: 365-370

Christophers S.R., 1933, The Fauna of British India, including Ceylon and Burma, Diptera Vol -V. Taylor and Francis London: pp.360

Devine G.J., and Furlong M.J., 2007, Insecticide use: Contexts and ecological consequences, Agr. Human. Values., **24:** 281-306 http://dx.doi.org/10.1007/s10460-007-9067-z

Ghosh A., Chowdhury N., and Chandra G., 2012, Plant extracts as potential mosquito larvicides, Indian. J. Med. Res., **135(5)**: 581-598

Jeyabalan D., Arul N., and Thangamathi P., 2003, Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston, Bioresource Technology, **89**: 185-189 http://dx.doi.org/10.1016/S0960-8524(03)00036-1

Kundu M., Rawani A., and Chandra G., 2013, Evaluation of mosquito larvicidal activities of seed coat extract of *Cassia sophera* L., J. Mosq. Res., **3(11)**: 76-81

Kamaraj C., Rahman A., Bagavan A., Zahir A., Elango G., Kandan P., Rajakumar G., Marimuthu S., and Santhoshkumar T., 2010, Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae), Trop. Biomed., **27(2)**: 211-219

Kamaraj C., Rahuman A., Bagavan A., Elango G., Zahir A., and Santhoshkumar T., 2011b, Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors, Asian. Pac. J. Trop. Med., 4(9): 698-705 http://dx.doi.org/10.1016/S1995-7645(11)60177-6

Kamaraj C., Bagavan A., Elango G., Zahir A.A., Rajakumar G., Marimuthu S., Santhoshkumar T., and Rahuman A., 2011a, Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*, Indian. J. Med. Res., **134**(1): 101–106

Lee S.E., Kim J., Lee S., 2001, Insecticide resistance in increasing interest, Agric Chem. Biotechno., **44**: 105–112

Ling Z.Q., Xie B.J., and Yang E.L., 2005, Isolation, characterization, and determination of antioxidative activity of oligomeric procyanidins from the seedpod of *Nelumbo nucifera* Gaertn, J. Agric. Food. Chem., **53**(7): 2441–2445 http://dx.doi.org/10.1021/jf040325p

Mukherjee P.K., 2002, Quality control of herbal drugs—an approach to evaluation of botanicals, 1st edn. Business Horizons, New Delhi, pp.604–608

Mukherjee P.K., Saha K., Das J., Pal M., and Saha B.P., 1997, Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera*, Planta. Med., **63(4)**: 367–369 http://dx.doi.org/10.1055/s-2006-957705

Macedo M., Consoli R.A.G.B., Grandi T.S..M, Des Anjos A.M.G., De Olivira A.B., Mendes M.M., Queiroz R.O., and Zani C.L., 1997, Screening of Asteraceae (Compositae) plant extract for larvicidal activity against *Aedes fluviatilis* (Diptera : Culicidae), Mem. Inst. Osw. Cruz., **92**: 565-570 http://dx.doi.org/10.1590/S0074-02761997000400024

Nayyar G.M.L., Breman J.G., Newton P.N., and Herrington J., 2012, "Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa", *Lancet*,. *Infect. Dis*., **12(6)**: 488–496 http://dx.doi.org/10.1016/S1473-3099(12)70064-6

Rawani A., Ghosh A., Laskar S., and Chandra G.. 2012, Aliphatic amide from seeds of *Carica papaya* as mosquito larvicide, pupicide, adulticide, repellent and smoke toxicant, J. Mosq. Res., **2(2)** doi:10.5376 /jmr.2012.02.0002.

Rawani A., Mallick H.K., Ghosh A., and Chandra G., 2009, Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae), Parasitol. Res**., 105(5)**: 1411-1417 http://dx.doi.org/10.1007/s00436-009-1573-z

Singha S., Adhikari U., Ghosh A., and Chandra G., 2012, Mosquito Larvicidal Potentiality of *Holoptelea integrifolia* Leaf Extract against Japanese Encephalitis Vector, *Culex vishuni* Group, J. Mosq. Res., **2(4)**: 25-31

Singha S., Adhikari U., and Chandra G., 2011, Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say, Asian. Pac. J. Trop. Biomed., 1(1): S119-S123 http://dx.doi.org/10.1016/S2221-1691(11)60137-8

Tabashnik B.E., 1994, Evolution of resistance to *Bacillus thuringiensis*, Annu. Rev. Entomol., **39**: 47-79 http://dx.doi.org/10.1146/annurev.en.39.010194.000403

Taylor W.R., Hanson J., Turner G.D., White N.J., and Dondorp A,M., 2012, Respiratory manifestations of malaria, Chest, **142(2)**: 492–505 http://dx.doi.org/10.1378/chest.11-2655

Wattal B.L., Joshi G.C., and Das M., 1981, Role of agriculture insecticides in precipitating vector resistance, J. Commun. Dis., **13**: 71-73

World Health Organization, 1981, Instructions for determining the susceptibility of resistance of mosquito larvae to insecticides, WHO/ VBC., **81**(807):1-6

Yu J., and Hu W.S., 1997, Effects of neferine on platelet aggregation in rabbits, **32**: 1–4